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Exploration of Binding and Toxic Site of Botulinum Neurotoxin.

Annual Report

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Planter nerves-lumbrical of the hind paw of the mouse was established as neuromuscular (NM) preparation for the first time for studying botulinum neurotoxin induced paralysis. Paralysis induced by dichain neurotoxin (type A or E) was delayed or antagonized if NM preparations (phrenic nerve-hemidiaphragm of the mouse) were incubated with isolted heavy chain prior to or during incubation of the dichain neurotoxin. This was the first direct demonstration of such competition using NM junction preparation and pharmacological activity.

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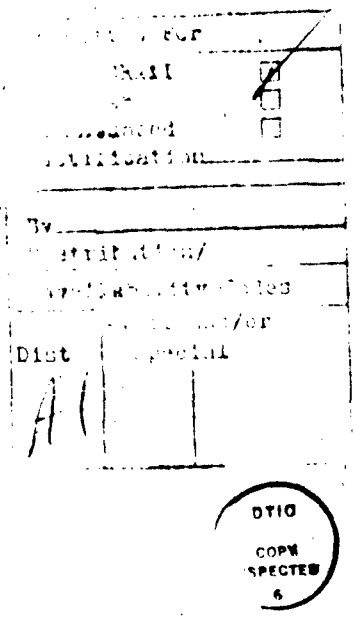
FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).



Summary

The dissection of the hind paws of a mouse provide nerve muscle preparation (Planter nerves-lumbrical muscle preparation). These are thin, short and flat. The location of neuromuscular junctions is highly predictable. These preparations were introduced for the first time for studying botulinum neurotoxin induced paralysis; primarily for electron microscopy of the binding of radiolabelled neurotoxin.

The heavy chain (M_r 97,000) isolated from type A neurotoxin (M_r 145,000) significantly antagonized the poisoning effect of the type A as well as type E neurotoxin. The heavy chain thus appeared to bind to the "receptors" on neuromuscular junction to which the whole neurotoxin binds. This was the first direct demonstration of such competition using neuromuscular junction preparation and pharmacological activity.

Body of the report (Text)

Research activities under the broad goals of the contract entitled "Exploration of binding and toxic sites of botulinum neurotoxin" were in three complementary areas; i) development of a system to observe by electron microscopy the binding of labelled (e.g. radioactive) neurotoxin (NT) to the presynaptic membrane of neuromuscular junction (NMJ) and the internalization of the NT and ii) determination of the role of the heavy and light chians of the NT in the neuroparalysis. The second area led us to a new finding; the heavy chain of the NT forms channels in lipid bilayer (see annual report Feb 84-July 85).

1. Work on development of lumbrical muscle preparation for the study of botulinum NT was completed. An abstract was submitted for presentation at the annual meeting of Federation of American Societies for Experimental Biology in April 1985. Preparation of a manuscript began to be submitted to a refereed journal for publication as a paper.

Conclusion: The dissection of the hind paws of a mouse provides at least 4, and sometimes as many as 8, nerve muscle preparations that are thin, short, and flat. The location of NMJ is highly predictable, hence well suited for observation with Nomarski interference phase contrast optics and for electron microscopy, and also for electrophysiology. The muscles have comparatively few fibers hence their response to experimental agents such as botulinum NT is rapid and complete. In our electron microscope studies of the NT we plan to use these preparations rather than the commonly used phrenic nerve-hemidiaphragm. The lumbrical preparations incubated with various concentrations of type A NT were paralyzed in NT concentration-dependent fashiun. The tissue also responded to type E NT trypsinized and not trypsinized demonstrating the expected more than 100 fold activation of the NT.

2. Binding site of the neurotoxin: The heavy chain (mol. wt. 97,000) isolated from the type A NT (mol. wt. 145,000) was tested. We asked is this the portion of the intact NT (mol. wt. 145,000) with which it binds to the presynaptic membrane NMJ? Prior exposure of a NMJ preparation (mouse hemidiaphragm) to the H chain significantly delayed the poisoning effect of the NT. An amount of NT, or its 10-fold dilution paralyzed NMJ preparations in ~80 min or ~135 min, respectively. Preincubation of the NMJ with the H chain delayed the poisoning effect of the higher concentration of NT from 80 min to 150 min, thus in effect causing at least 90% inhibition. The heavy chain from type A NT also competed against the type E NT. The heavy chain thus appeared to bind to the "receptors" on the NMJ to which the whole NT binds. This was the first direct demonstration of such competition using NMJ preparation and pharmacological activity.

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